

## SHORT COMMUNICATION

### A new approach to examining scorpion peg sensilla: the mineral oil flood technique

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**Abstract.** All scorpions possess jointed, ventral appendages called pectines. These organs have chemosensory, peg-shaped sensilla that detect substrate-borne chemicals. Previous physiological studies show that neurons within peg sensilla respond to an assortment of volatile organic chemical stimulants blown across the sensillar opening. We developed an improved method of chemical stimulant delivery called the mineral oil flood technique to further investigate the neural circuitry of scorpion pectines. The new mineral oil flood technique allows us to deliver chemical stimulants directly to individual sensilla by introducing a polar, liquid substance under non-polar mineral oil. Unlike previous methods of stimulant delivery, the mineral oil flood technique allows for precise control over the duration of direct contact between a liquid stimulant of known concentration and a sensillum.

**Keywords:** Pectines, chemosensory, electrophysiology, stimulant, Scorpiones

Pectines are movable sensory appendages that extend from the mid-ventral surface of all scorpions (Cloudsley-Thompson 1955). They are similar in function to the antennae of mandibulate arthropods that detect airborne chemicals (Kaissling 1987; Itagaki & Hildebrand 1990), except that pectines are ground-directed and respond to substrate-borne chemicals. When scorpions move in their environment, the pectines sweep intermittently against the substrate to detect food (Krapf 1986; Skutelsky 1995) and pheromones (Gaffin & Brownell 1992, 2001).

The primary sensory elements on pectines are hundreds of minute structures called peg sensilla, which adorn the ground-facing surface of each pectinal tooth (Carthy 1966, 1968; Ivanov & Balashov 1979; Foelix & Müller-Vorholt 1983). Each peg sensillum has a single slit-like pore that allows chemical stimulants access to receptor neurons inside the peg shaft. Approximately 10 sensory neurons innervate each peg (Foelix & Müller-Vorholt 1983), and synaptic contacts exist between neurons within a sensillum (Gaffin & Brownell 1997a).

Peg sensilla respond with different neural activity patterns to alcohols, aldehydes, ketones, and esters (Gaffin & Brownell 1997b). The sensilla were stimulated indirectly through puffs of stimuli blown across entire peg fields (Gaffin & Brownell 1997b) or by static clouds of volatile organic compounds brought near the peg tips (Gaffin & Walvoord 2004). Although these methods of stimulant delivery elicit neural responses, they have limitations. Most importantly, it is impossible to introduce a stimulant to a single peg sensillum without stimulating its neighbors. There is also no way, as such, to tell if stimulating a neighboring sensillum influences the response of the recorded sensillum. In addition, the concentration of stimulant reaching the sensillar pore is unknown, and removal of the stimulant from the peg field is uncontrollable.

To overcome these limitations, we developed an improved method of chemical stimulus delivery called the mineral oil flood technique, which uses non-polar mineral oil as a medium for delivering polar, liquid stimulants to an individual sensillum. In this study, we describe our new method and compare spontaneous and chemically-induced neural activity of peg sensilla in air and under oil.

Mature female *Paruroctonus utahensis* (Williams, 1968) (Scorpiones: Vaejovidae) collected from Crane County (31°28'59"N, 102°40'38"W), Texas, in March of 2008 were the animals used for this study. We individually housed each scorpion in 3.8 l glass jars containing approximately 250 ml of sand from the scorpion's collection site. The animals were on a 15:9 h L:D cycle and kept in a room with a steady temperature and relative humidity (22° C, RH

55–60%). We fed each scorpion one early instar cricket biweekly and watered the sand of individual containers with 5 ml of deionized water twice a week. At the conclusion of our study, we deposited a voucher specimen in the Sam Noble Oklahoma Museum of Natural History (OMNH-16279).

For preparing a scorpion for electrophysiological study, we briefly anesthetized a live animal by cooling it for two minutes inside a freezer at –5° C. Then we immobilized the animal with modeling clay on a microscope slide (7.62 × 2.54 cm), ventral side up to expose the pectines. We positioned a modified cover glass (5 × 18 mm) with walls of wax approximately 1 mm high caudal to where the pectines join the body (Fig. 1a). A notch in the wax wall allowed access of a pecten to the chamber (Fig. 1b). We secured the pecten spine and teeth to the cover glass with double-sided adhesive tape and fine application of less than 5 µl of quick-drying adhesive glue (Instant Krazy Glue™). Next, we applied additional wax to the area where the pecten spine crossed the edge of the cover glass to complete the wall of the chamber. We then placed one drop of mineral oil (≈5 µl) over the pecten with a 0.25 ml syringe (Fig. 1c). We secured the animal onto an adjustable platform and located peg fields with a high-powered compound microscope equipped with epi-illumination (Olympus BX50-WI). Lastly, we used an electrolytically-sharpened tungsten electrode to make extracellular recordings of chemosensitive neurons within individual sensilla (Gaffin & Brownell 1997b).

To record peg neurons, we digitized electrical activity with an analog to digital converter (1401-plus digitizing hardware, CED, Cambridge, England) and analyzed the record using Spike 2 laboratory software (CED, Cambridge, England). Impulses ("spikes") from each spontaneously active chemosensitive neuron were identified and separated into three classes (A1, A2, and B), based on the characteristic spike waveforms of the impulses from each neuron (Gaffin & Brownell 1997b). We used auto-correlation analysis to determine the purity of each spike class and cross-correlation analysis to detect synaptic interactions among spike classes (for details on auto- and cross-correlation analyses, see Gaffin & Brownell 1997a; Eggermont 1990).

To stimulate peg sensilla chemically, we filled a glass capillary tube (stimulant pipette) with an approximately 10 µm diameter tip with 95% ethanol (Gaffin & Walvoord 2004). We used a nonmetallic syringe needle (World Precision Instruments, Inc. MicroFil™ MF34G) to transfer the ethanol to the stimulant pipette. We then placed the stimulant pipette into a glass electrode holder positioned on a mechanical micromanipulator (Sutter Instrument Corp. 1140). For fluid stimulant introduction, we maneuvered the pipette tip with

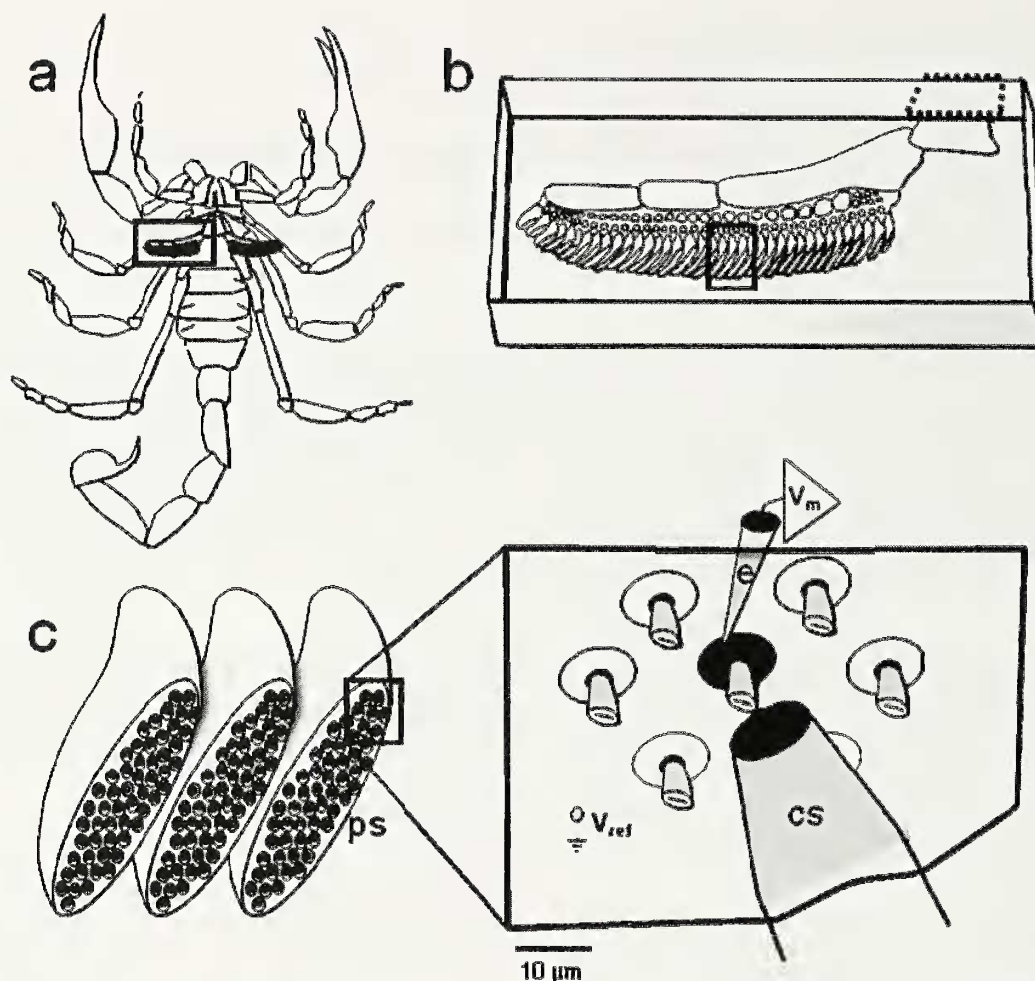


Figure 1.—Scorpion pecten configuration. a. The right pecten (outlined) as positioned for electrophysiological examination. b. Close-up view of the right pecten in a stimulation chamber. A piece of the wax barrier (dashed outline) is removed for pecten placement. c. Left, an expanded field of view of patches of peg sensilla (ps). Right, a mineral oil overlay of an expanded field of view of a peg field. A microelectrode (e) is inserted at the base of a single peg, under oil, to record baseline neural activity in the presence or absence of a chemical stimulant (cs).

the micromanipulator so that it touched the pore of the recorded sensillum. Fig. 1c shows the general configuration of the peg field, microelectrode, and chemical stimulus delivery device during chemical stimulation.

In general, we were able to record spontaneous neural activity under oil for extended periods, some longer than six hours. Because the stability of a recording often depended on the animal's inability to move its pecten, we improved the method of adhering the pecten to the cover glass. The most effective method was careful application of quick-drying adhesive to the pecten spine and the distal-lateral surface of each pectinal tooth. The least effective adhesives were paraffin wax and silicone gel.

Because the spread of mineral oil beyond the pecten and onto the animal's body induced pecten movement, we assembled a barrier between an isolated pecten and the rest of the animal. The most useful barriers were about one millimeter high, which still allowed easy access of an electrode and stimulant pipette to the sensillum, while preventing the spread of mineral oil beyond the pecten. The amount of mineral oil applied to the pecten also affected the quality of a preparation. Volumes of oil greater than or equal to 50  $\mu$ l were not contained within the barrier. Excessive oil also blurred the field of view. In contrast, 5  $\mu$ l of oil actually improved the resolution of the peg field; we could discern individual peg sensillar shafts, which is difficult when viewing sensilla in air. In addition, 5  $\mu$ l of oil remained

within the barrier and provided sufficient overlay for stimulant introduction.

The presence of mineral oil on peg sensilla did not affect baseline neural activity. Fig. 2 compares the spontaneous firing pattern of a peg sensillum in air with that of another peg sensillum under oil. Cross-correlation analyses reveal the same synaptic interactions in each record: when spike B fired, it inhibited spikes A1 and A2 for approximately 0.1 s.

Using the mineral oil flood technique, we confined chemical stimulation to a single peg sensillum and controlled the onset and removal of the stimulant. For example, the right panel of Figure 3 shows the introduction and removal of liquid ethanol to a peg sensillum under oil for durations of one, two, and three seconds. Stimulations were consecutive and spaced approximately 20 s apart, which produced receptor adaptation in the third response. In contrast, the left panel of Fig. 3 shows a prolonged neural recovery after introduction of a drop of ethanol to a peg sensillum in air. The time-expanded view of stimulant introduction shows the extent of record disturbance; recorded electrical activity was inconsistent across all stimulations.

Our study represents the first account of selective chemical stimulation of individual scorpion peg sensilla with a known concentration of aqueous stimulant. Because the presence of mineral oil over a sensillum did not affect the baseline neural activity, we used



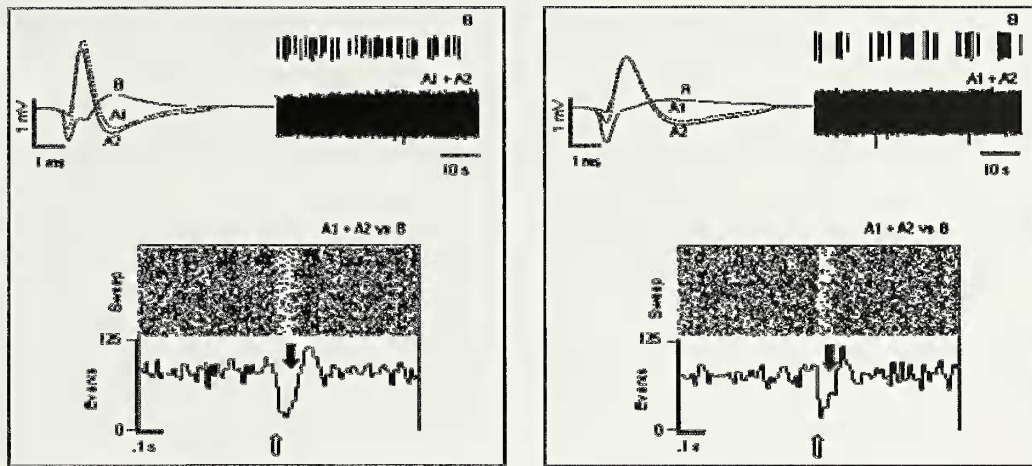


Figure 2.—Processed recordings of spontaneous neural activity. Shown are recordings of chemosensitive neurons (B, A1, and A2) from a sensillum in air (left panel) and a different sensillum under oil (right panel). Each line represents a neural impulse within the parsed 50 s record. The enlarged figures at the left of each recording indicate the B, A1, and A2 impulse waveforms. Cross-correlograms reveal that the firing of B (white arrow) inhibits the activity of A1 and A2 (black arrow) both in air and under oil.

oil as a medium through which to directly stimulate individual pegs with 95% ethanol for controlled durations.

One limitation of the mineral oil flood technique is that we can only use polar liquids as stimulants. Non-polar stimulants would mix with non-polar mineral oil. Therefore, future studies on peg sensillar function will use varying concentrations of polar solutions, such as salts and organic compounds that contain fewer than three carbons as stimulants.

In forthcoming experiments, we will use the mineral oil technique to further our understanding of peg sensillar function. This new method should generate the quantifiable data necessary for comparing sensillar neural responses. For example, we aim to stimulate many peg sensilla individually to compare response intensities to varying concentrations of stimulants. This will help us determine if all peg sensilla are functionally equivalent and if they follow dose-dependent response patterns. Additionally, no studies to date have tested for possible peg-to-peg interactions. We plan to test for synaptic interactions between neurons of neighboring sensilla by stimulating one peg sensillum while recording electrophysiologically from a neighboring sensillum. If synaptic interactions extend beyond neurons of an individual sensillum, we should observe a change in neural

activity of the recorded sensillum as we stimulate its neighbor. Such a situation would provide evidence of lateral inhibition, which is a form of peripheral processing seen most commonly in the vertebrate retina.

#### ACKNOWLEDGMENTS

We thank Drs. Mariëlle Hoefnagels, Don Wilson, and Ari Berkowitz for their helpful suggestions and use of equipment. We also thank Nataliya Popokina for assistance in collecting and maintaining our animals. Finally, we thank the Life Fund of the University of Oklahoma Foundation and the OU Zoology Adams Scholarship Fund for providing support for this work.

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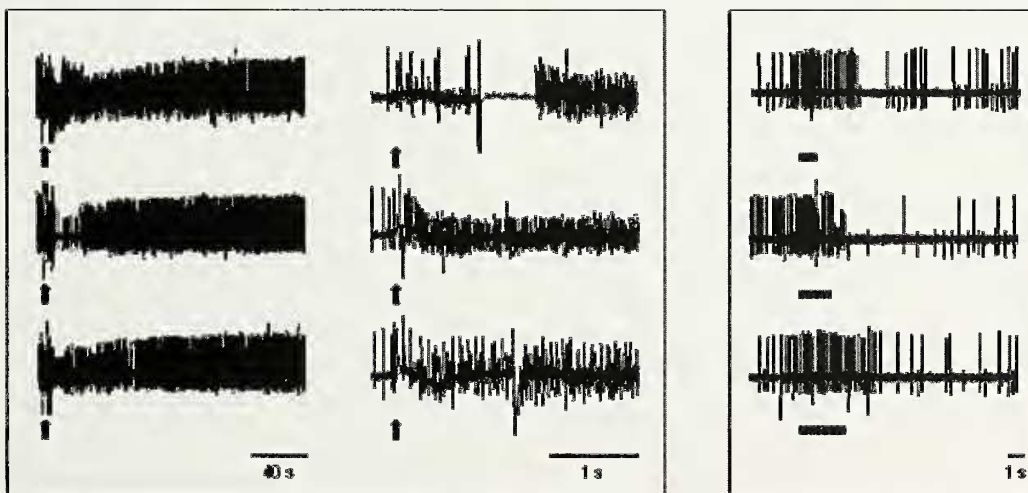


Figure 3.—Unprocessed recordings of neural activity during chemical stimulation. In air (left panel), fluid ethanol was introduced to a peg sensillum at three successive occasions (arrows). To the right of each record is a time-expanded view of the exact moment of stimulant contact with the recorded sensillum. Under oil (right panel), fluid ethanol was reversibly applied to a peg sensillum for 1, 2, or 3 s (horizontal bars).

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*Manuscript received 30 September 2008, revised 4 May 2009.*